REPTILES & AMPHIBIANS

Twinning in a Wild Breeding Population of Gharials (*Gavialis gangeticus*) in India

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Twinning is defined as the development of two embryos in a single egg (Yntema 1970) and has been reported in turtles (Fujiwara 1964; Louro and Pereira 2009), lizards (Speer and Bayless 2000), and snakes (Manimozhi et al. 2006; Dolia 2018), as well as crocodilians. Examples of twinning in crocodilians include *Alligator mississippiensis* (Ferguson 1985), *Caiman latirostris* (Larriera and Imhoff 1994), *Crocodylus acutus* (Velasco 2010), *C. intermedius* (Thorbjarnarson 1989), *C. johnstoni* (Ferguson 1985), *C. niloticus* (Blomberg 1979; Ferguson 1985), *C. porosus* (Hibberd 1996), *C. moreletii* (Platt et al. 2000), *C. siamensis* (Platt et al. 2012), *C. palustris* (Sharma et al. 2020), and *Gavialis gangeticus* (Acharjyo and Singh 1989). Twinning in reptiles has been observed both in captivity (Acharjyo and Singh 1989; Di Marzio et al. 2023) and in nature (Sharma et al. 2020). However, few reports document twinning in wild reptilian populations, likely attributable to methods of examination, large clutch sizes, and synchronous hatching, especially in communally nesting species (Piovano et al. 2011; Di Marzio et al. 2023).

Twins in an egg usually start development at the same time, but can result in hatchlings of equal or unequal sizes, or one developed and one in an embryonic phase (Hirasawa et al. 2019). In crocodiles, twinning occurs as twins, double yolks, or axial bifurcation (Ferguson 1985). One-egg twins are usually undersized compared to normal hatchlings due to the competition for resources inside the egg (Acharjyo and Singh 1989; Platt et al. 2000), which can also compromise post-hatching survival. Causes of twinning are not known but some assumed mechanisms have been reported. Low genetic variation in bottlenecked populations can result in an

Figure 1. Twin Gharial (*Gavialis gangeticus*) hatchlings removed from the egg in which they were found; note their small size and the shared yolk sac. Photograph by Shantanu Ugemuge.

Figure 2. A second incidence of twinning in the Gharial (*Gavialis gangeticus*), with an undeveloped embryo still attached to the hatchling via the umbilical cord. Photograph by Shantanu Ugemuge.

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increased number of anomalies including twinning (Gautschi et al. 2002); however, females tend to be not genetically predisposed to produce twins (Eckert 1990). Exposure of eggs to high incubation temperatures for long durations (e.g., climate change and frequent heat waves) can lead to developmental abnormalities in reptiles in the wild (Telemeco et al. 2013). In captivity, abnormal fluctuations in temperature and humidity, infections, and improper amounts of oxygen during incubation can increase the frequency of twinning (Frye 1991; Di Marzio et al. 2023). Exposure to some chemicals and higher concentrations of chemical contaminants can lead to an increase in developmental abnormalities (Shirose et al. 1995; Velasco 2010). Twinning also has been related to the age and size of the females (Ferguson and Joanen 1983; Tucker and Janzen 1997).

Herein we report a case of twinning in a wild breeding population of the Gharial (*Gavialis gangeticus*) in the Girwa River, Katerniaghat Wildlife Sanctuary, Uttar Pradesh, India. On 17 June 2023, we found an unhatched egg in a nest that had been excavated by a female Gharial. The unhatched egg was opened manually, and we recovered two hatchlings. Both were alive, of approximately similar size, and were connected by an umbilical cord (Fig. 1); both were weak and thin compared to the other hatchlings; and the yolk sac was visibly smaller and reduced in volume compared to those in eggs housing single hatchlings. We also observed a second egg with a possible case of twinning in a separate clutch during the same breeding season when we found a developed hatchling with an undeveloped embryo attached via an umbilical cord (Fig. 2). During the 2019 nesting season, we also had encountered a Gharial egg with two developed hatchlings, both of which were smaller than other hatchlings in the nest and both died within a day of their hatching and release into the water. A previous report of twinning in the Gharial (Acharjyo and Singh 1989) described two hatchlings of unequal size, the smaller of which was dead.

We have been monitoring Gharial nests in Katerniaghat since 2016 and these are the only cases of twinning that we have encountered. They appear to have no substantive effect on the nesting success of the breeding population, in which annual nest numbers have ranged between 19 and 36 in the last five years with hatching success rates reaching over 90%. However, the low frequency of twinning could reflect poor detection rates as most of the females nest communally and excavate the majority of the nests on their own.

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